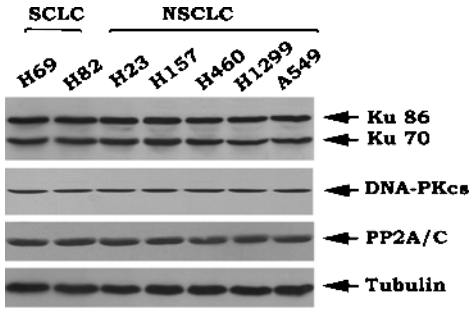

Figure W1. Expression of Ku 70, Ku 86, DNA-PKcs, and PP2A in human lung cancer cells. Expression levels of endogenous Ku 70, Ku 86, DNA-PKcs, and PP2A/C in human lung cancer cells were analyzed by Western blot using Ku 70, Ku 86, DNA-PKcs, or PP2A/C antibody, respectively. Tubulin was used as a loading control.

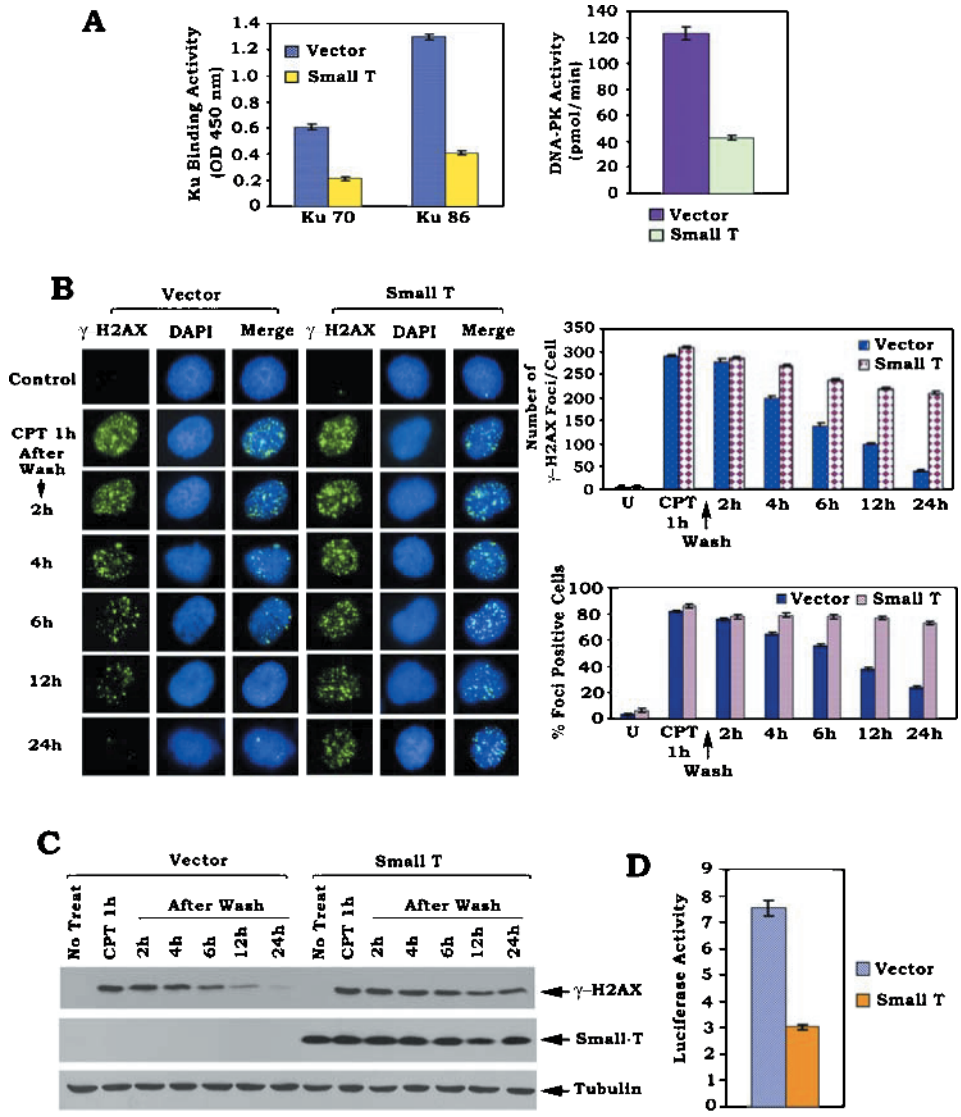

Figure W2. Disruption of PP2A activity by expression of small T antigen downregulates Ku and DNA-PK activity in association with suppression of DNA end-joining and DSB repair. (A) Ku DNA binding activity or DNA-PK activity was measured in H1299 cells expressing small T or vector-only control using a Ku 70/86 DNA Repair Kit or a SignaTECT DNA-PK Assay Kit, respectively. Data represent the mean ± SD of three separate determinations. (B) H1299 cells expressing small T or vector-only control were treated with CPT (5 μM) for 1 hour. Then, cells were washed three times and incubated in normal culture medium for various times up to 24 hours. DSBs were determined by analysis of γ-H2AX by immunostaining or Western blot. The number of γ-H2AX foci per cell was determined on a cell to cell basis by the quantitative analysis of at least 30 randomly chosen cells as described [1]. The percentage of γ-H2AX foci-positive cells was determined by analyzing 100 randomly chosen cells as described [2]. (C) H1299 cells expressing small T or vector-only control were treated as previously mentioned. γ-H2AX was analyzed Western blot using a γ-H2AX antibody. (D) DNA end-joining activity was measured in H1299 cells expressing small T or vector-only control as described in the Materials and Methods section. Data represent the mean ± SD of three separate determinations.

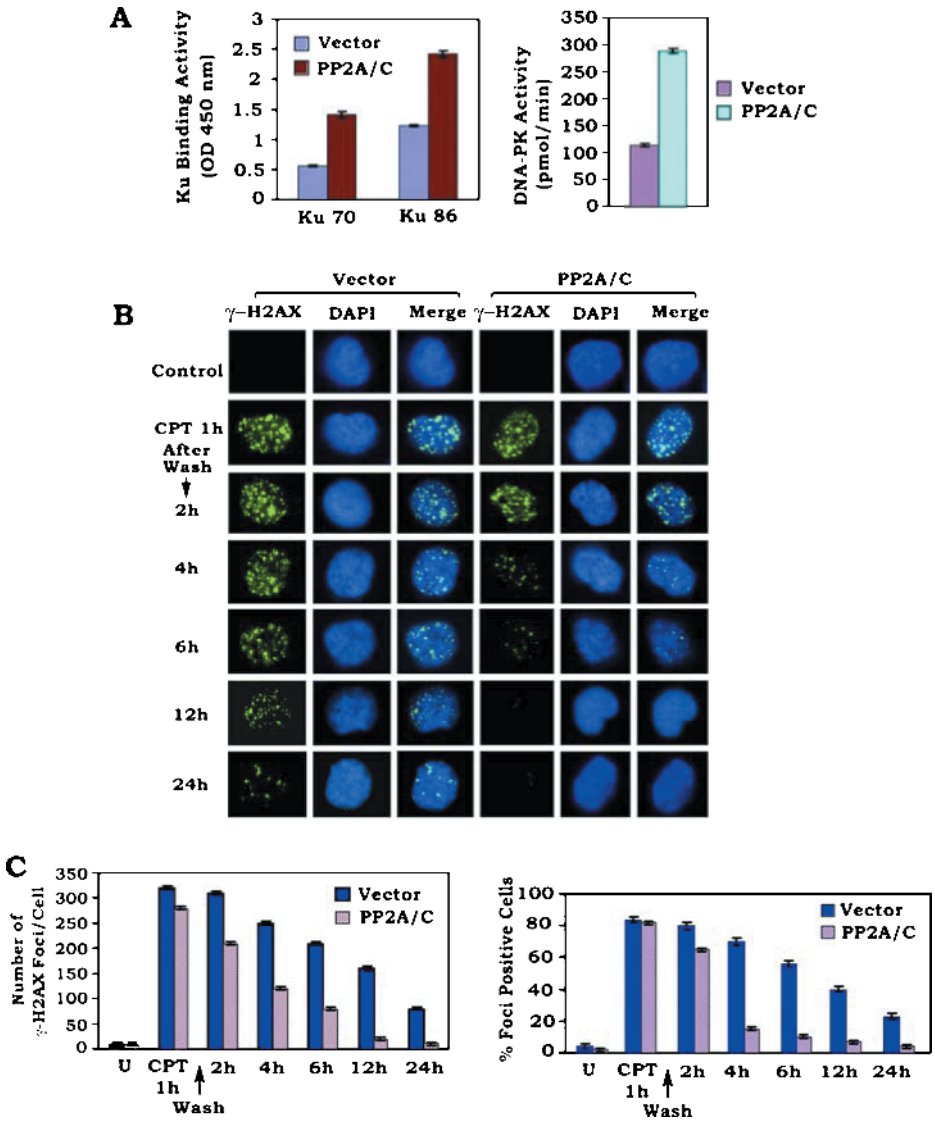

Figure W3. Overexpression of PP2A upregulates Ku and DNA-PK activities leading to accelerated DSB repair. (A) Ku DNA binding activity or DNA-PK activity was measured in H1299 cells overexpressing HA-PP2A/C or vector-only control using a Ku 70/86 DNA Repair Kit or a SignaTECT DNA-PK Assay Kit, respectively. Data represent the mean ± SD of three separate determinations. (B and C) H1299 cells overexpressing HA-PP2A/C or vector-only control were treated with CPT (5 μM) for 1 hour. Cells were washed three times and incubated in normal culture medium for various times up to 24 hours. DSBs were determined by analysis of γ-H2AX by immunostaining. The number of γ-H2AX foci per cell was determined on a cell to cell basis by the quantitative analysis of at least 30 randomly chosen cells as described [1]. The percentage of γ-H2AX foci-positive cells was determined by analyzing 100 randomly chosen cells as described [2].

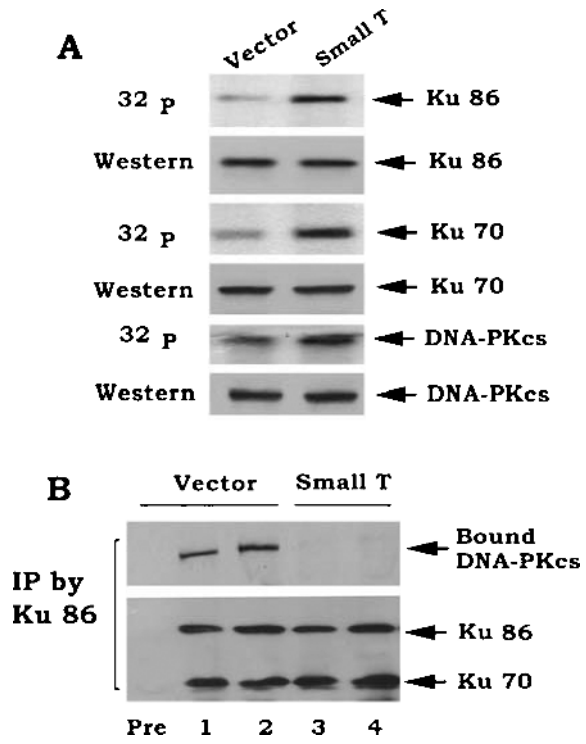

Figure W4. Expression of small T results in increased phosphorylation of Ku 70, Ku 86 and DNA-PKcs, which leads to dissociation of Ku/DNA-PKcs complex. (A) H1299 cells overexpressing small T or vector-only control cells were metabolically labeled with ^{32}P-orthophosphoric acid for 90 minutes. Ku 70, Ku 86, or DNA-PKcs was immunoprecipitated and phosphorylation was determined by autoradiography. (B) H1299 cells expressing small T or vector-only control cells were disrupted in EBC lysis buffer. CoIP was performed using Ku 86 antibody. The Ku-associated DNA-PKcs, Ku 70, and Ku 86 were then analyzed by Western blot. Rabbit preimmune serum (Pre) was used as a control.

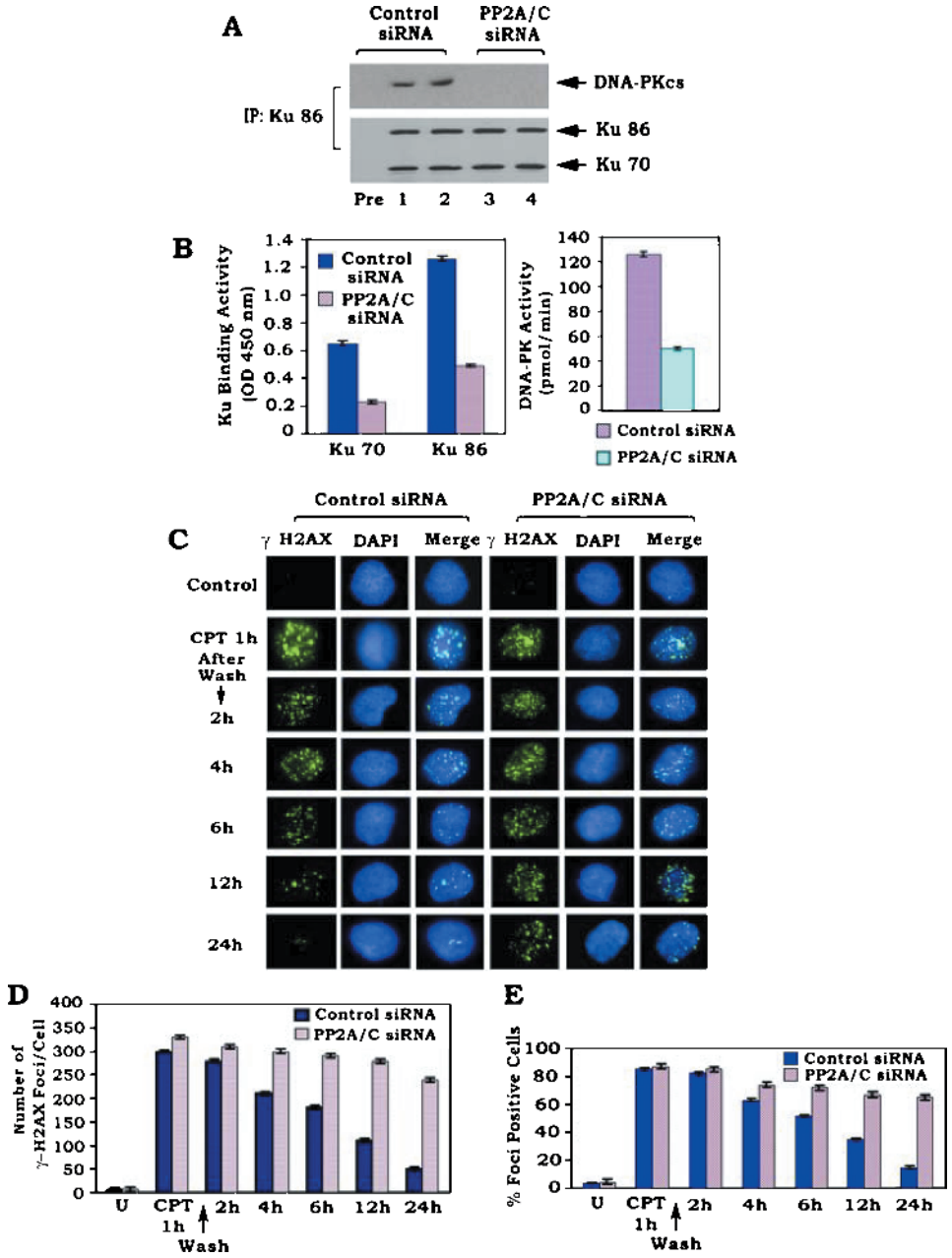

Figure W5. Depletion of PP2A/C by RNAi results in disruption of the Ku/DNA-PKcs complex and suppression of Ku and DNA-PK activities leading to decreased DSB repair. (A) H1299 cells expressing PP2A/C siRNA or control siRNA were disrupted in EBC lysis buffer. CoIP was performed using a Ku 86 antibody. The Ku-associated DNA-PKcs, Ku 70, and Ku 86 were then analyzed by Western blot. Rabbit preimmune serum (Pre) was used as a control. (B) Ku DNA binding activity or DNA-PK activity was measured in H1299 cells expressing PP2A siRNA or control siRNA using a Ku 70/86 DNA Repair Kit or a SignaTECT DNA-PK Assay Kit, respectively. Data represent the mean ± SD of three separate determinations. (C and D) H1299 cells expressing PP2A/C siRNA or control siRNA were treated with CPT (5 μM) for 1 hour. Cells were washed three times and incubated in normal culture medium for various times up to 24 hours. DSBs were determined by analysis of γ-H2AX by immunostaining. The number of γ-H2AX foci per cell was determined on a cell to cell basis by the quantitative analysis of at least 30 randomly chosen cells as described [1]. (E) The percentage of γ-H2AX foci-positive cells was determined by analyzing 100 randomly chosen cells as described [2].

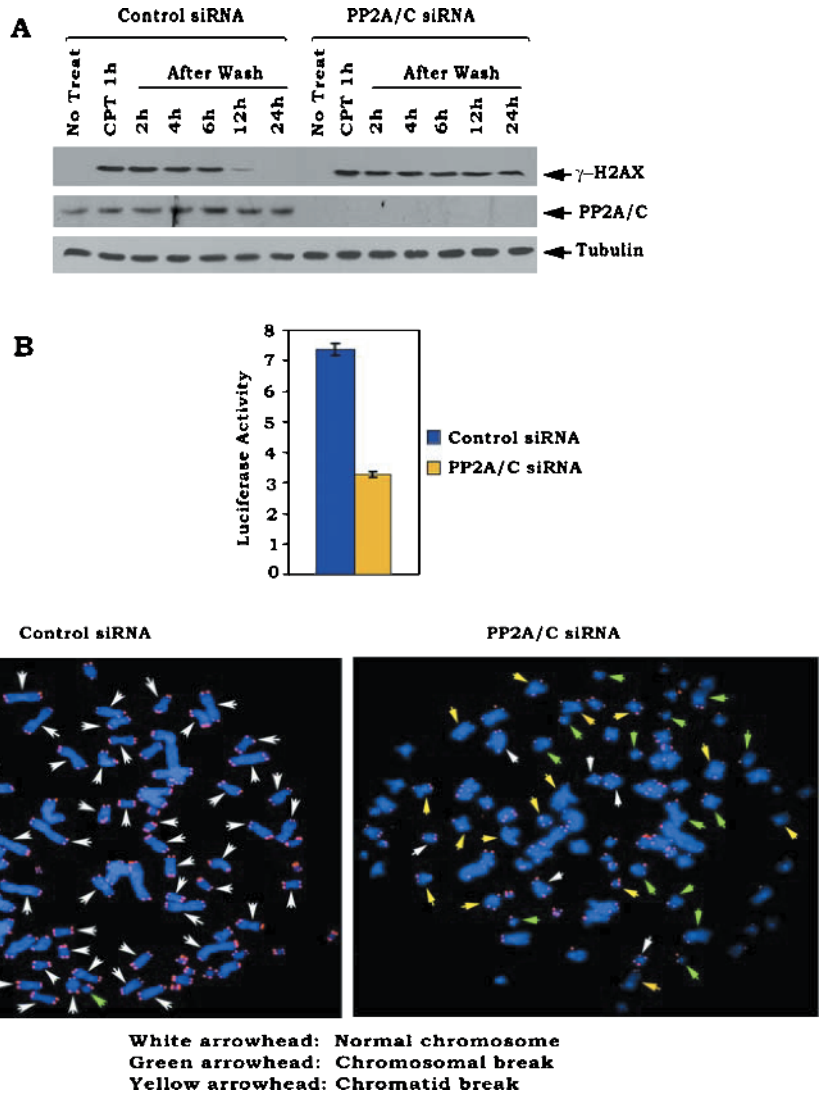

Figure W6. Specific knockdown of PP2A/C suppresses DSB repair and DNA end-joining leading to genetic instability. (A) H1299 cells expressing PP2A/C siRNA or control siRNA were treated with CPT (5 μM) for 1 hour. Cells were washed three times and incubated in normal culture medium for various times up to 24 hours. γ-H2AX was determined by Western blot using a γ-H2AX antibody. (B) DNA end-joining activity was measured in H1299 cells expressing PP2A/C siRNA or control siRNA as described in the Materials and Methods section. Data represent the mean ± SD of three separate determinations. (C) Cytogenetic abnormalities were analyzed by T-FISH in H1299 cells expressing PP2A/C siRNA or control siRNA. DAPI-stained chromosomes are blue. Red dots come from telomere signals. Color-coded arrowheads indicate a normal chromosome and different kinds of cytogenetic abnormalities (white indicates normal chromosome with four telomere signals; green, chromosomal break with two telomere signals; yellow, chromatid break with three telomere signals).

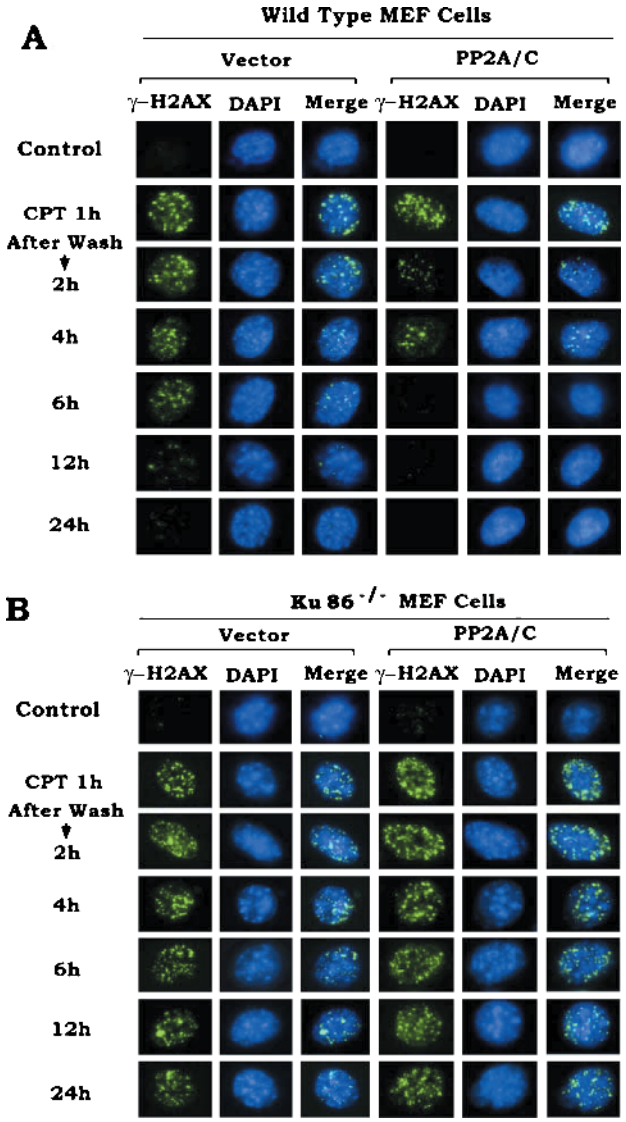

Figure W7. Ku 70 and Ku 86 are required for PP2A promotion of DSB repair. (A and B) Wild type or Ku $86^{-/-}$ MEF cells overexpressing HA-PP2A/C or vector-only control were treated with CPT (5 μM) for 1 hour. Then, cells were washed three times, and incubated in normal culture medium for various times up to 24 hours. DSBs were determined by analysis of γ-H2AX foci. The number of γ-H2AX foci per cell was determined on a cell to cell basis by the quantitative analysis of at least 30 randomly chosen cells as described [1]. The percentage of γ-H2AX foci-positive cells was determined by analyzing 100 randomly chosen cells as described [2].